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Exploitation of Apiaceae family essential oils as potent biopesticides and rich source of phellandrenes

Epameinondas Evergetis[a], Antonios Michaelakis[b], Serkos A. Haroutounian[a,*]

[a] Chemistry Laboratory, Agricultural University of Athens, Iera odos 75, Athens 11855, Greece
[b] Benaki Phytopathological Institute, 8 S. Delta Str., Kifissia, Athens 14561, Greece

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ABSTRACT

The chemical content and insecticidal activities of essential oils (EOs) derived from various Apiaceae (Umbeliferae) family taxa, including the most representative coastal (*Malabaila aurea* and *Echinophora tenuifolia* ssp. *sibthorpiana*), mountainous (*Cachrys ferulacea* and *Chaerophyllum aromaticum*) and cultivated (*Anethum graveolens*) specimens, were determined. The chemical analyses results herein showed that the EO of *A. graveolens* specimen contains a major component, particularly high amounts of α-phellandrene (59%) indicating that this widely cultivated plant may account as a novel-alternative industrial source for this molecule. The insecticide properties of the EOs studied – and their most abundant compounds – were evaluated against the *Culex pipiens* L. larvae of 3rd and early 4th instars. Results indicated that many of them display noteworthy toxicity, with the EO of *A. graveolens* being the most active, displaying an LC_{50} value of 52.74 mg L^{-1}. The EOs of *E. tenuifolia* ssp. *sibthorpiana* and *C. aromaticum* were also highly active exhibiting LC_{50} values near 60 mg L^{-1}. Among the tested oils, the EO of *A. graveolens* exhibits noteworthy potentials for use either as bioremediation cultivar in mosquito-thriving areas and/or the development of novel biocides for the control of mosquitoes.

1. Introduction

The eternal struggle of mankind against insects throughout history has faced both glorious victories and disgraceful defeats. Mosquitoes, besides being the most annoying group of all blood-sucking arthropods, they also act as transmitters of various serious diseases with severe and diverse social and financial consequences. Thus, the struggle against mosquitoes has become the favorite target of this fight, emerging as the field of the most controversial victories of mankind against insects. The large-scale intervention with synthetic pesticides, although effective, consists a major threat for public health (Kaushik and Kaushik, 2007; Ray and Fry, 2006), since their use has resulted in the development of resistant mosquito populations (Hemingway et al., 2004) that generated a series of intercrossing economical and environmental (Berticat et al., 2008; Lee and Wang, 2006) impacts. Nowadays, the mosquito insecticides are considered as one of the major and most persistent environmental pollutants while the EC legislative ban of numerous pesticides (Directive 98/8/EC) generated a critical need for the development of novel, more potent, mosquito control agents. The focus of relative research is re-oriented to include the development of new, safer products which display the ability to control efficiently the mosquito population and eventually detent the spread of their corresponding vector diseases. In this respect, various fungi, bacteria, insects, fishes, their mixed interventions and plant natural products have been considered and tested as mosquito control agents (Becker et al., 2003).

Main objective of this study is to contribute in the effort to develop novel, environmentally sound alternatives for mosquito jugulating. The results herein may also contribute toward the minimization and/or elimination of the persistent organic pollutants (POPs) inflow among rural and urban habitats. In this respect, we have considered the essential oils (EOs) as the natural products of choice, because they congregate a combination of crucial and highly desirable characteristics, such as fast degradation and low toxicity to non-target insects and animals. The EOs effectiveness is evaluated on mosquito larvae, since larviciding is considered as the principal method to control most *Culex* species breed in confined breeding sites (e.g., small pools, septic tanks, containers), especially in urban and semi-urban areas.

The Apiaceae (Umbeliferae) family was selected as the EOs plant source, relying on previous research reports which established that the EOs of various Apiaceae plants exhibit potent larvicidal activities against mosquitoes (Evergetis et al., 2009, in press). Thus, we have assessed the chemical content and biocide activities of the EOs derived from a broad spectrum of the family's biodiversity taxa, including the most representative coastal (*Malabaila aurea* and *Echinophora tenuifolia* ssp. *sibthorpiana*), mountainous (*Cachrys*

* Corresponding author.
E-mail address: sehar@aua.gr (S.A. Haroutounian).

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Table 1
Collection data.

Species	Abbreviation	Vegetative stage	Date	Location
Anethum graveolens	*AG*	Flowering	20/07/03	Mt. Parnassos, continental Greece
Echinophora tenuifolia ssp. *sibthorpiana*	*ET*	Flowering	14/07/03	Mt. Madara, Crete island
Chaerophyllum aromaticum	*CA*	Flowering	05/06/03	Mt. Oiti, continental Greece
Cachrys ferulacea	*CF*	Before flowering	05/06/03	Mt. Madara, Crete island
Malabaila aurea	*MA*	Flowering	14/07/03	Mt. Parnon, continental Greece

ferulacea and *Chaerophyllum aromaticum*) and cultivated (*Anethum graveolens*) specimens. In addition, the biocide activities of some phytochemicals commonly found in Apiaceae plants, such as α-phellandrene, *cis*-ocimeme, β-myrcene and bornyl acetate that have not previously screened against *Culex* mosquitoes. Both α-phellandrene and *cis*-ocimeme constitute the major compounds of *A. graveolens* and *E. tenuifolia* ssp. *sibthorpiana* EOs, respectively), while β-myrcene and bornyl acetate are considered as the minor constituents of most EOs.

Currently, α-phellandrene has been isolated from *Eucalyptus* species and found toxic against two *Aedes* species (Cheng et al., 2009). It is noticeable that the EO derived from *A. graveolens* plant contains as major constituent the molecule of α-phellandrene (59%), indicating that the investigated variation of this cultivated Apiaceae plant may account as a potential industrial source of this well known monocyclic unsaturated sesquiterpene that contains two endocyclic double bonds.

2. Materials and methods

2.1. Plant material

Five different taxa of the Apiaceae family, Apioideae subfamily, belonging to five tribes and five different genera were collected for this study. In particular, the following representative species were investigated: Echinoforeae tribe the *E. tenuifolia* ssp. *sibthorpiana* (Guss.) Tutin, Peucedaneae tribe the *A. graveolens* L., Scandiceae tribe the *C. aromaticum* L., Smyrnieae tribe the *C. ferulacea* (L.) Calestani and Tordylieae tribe the *M. aurea* (Sibth. and Sm.) Boiss., an endemic in Balkans taxon (Pimenov and Leonov, 1993; Tutin et al., 1968). All species grow naturally in Greece and their full collection details are provided in Table 1. *A. graveolens* L., as it originates in India and SW Asia, was found to grown in a vineyard. This population had profoundly escaped cultivation and naturalized. A voucher specimen of each plant collected has been deposited in the herbarium of the Agricultural University of Athens, Athens, Greece.

2.2. Isolation of the essential oils

The freshly collected plant materials (stems, leaves and flowers) were washed thoroughly, chopped off finely and subjected to steam distillation in a Clevenger-type apparatus with 3 L of H_2O and using a microwave accelerated reaction system (MARS 5) for 40 min at 1400 W. The obtained EOs were dried over anhydrous sodium sulfate and stored at 4 °C. The EO yield of each plant is included in Table 2.

2.3. Chromatographical analyses

Gas chromatography (*GC*). All GC analyses were carried out on a Agilent Technologies 7890A gas chromatograph, fitted with a HP 5MS 30 m × 0.25 mm × 0.25 μm film thickness capillary column and FID. The column temperature was programmed from 60 to 280 °C at a initial rate of 3 °C/min. The injector and detector temperatures were programmed at 230 and 300 °C, respectively. Helium was used as the carrier gas at a flow rate 1 mL/min.

Gas chromatography–mass spectrometry (*GC–MS*). The GC–MS analyses were performed on the same instrument connected with the Agilent 5957C, VL MS Detector with Triple-Axis Detector system operating in EI mode (equipped with a HP 5MS 30 m × 0.25 mm × 0.25 μm film thickness capillary column) and helium as the carrier gas (1 mL/min). The column was heated gradually from its initial temperature (60 °C) to 280 °C with a 3 °C/min rate. The compounds identification was based on comparison of their retention indices (RI) obtained (Van den Dool and Kratz, 1963) as compared to various n-alkanes (C9–C24). In addition, their EI-mass spectra were compared with the NIST/NBS and Wiley library spectra and the literature (Adams, 1995; Massada, 1976). Finally, the identity of the indicated phytochemicals was confirmed by comparison with available authentic samples.

2.4. Mosquito rearing

Mosquitoes were obtained from a colony of *Culex pipiens* biotype *molestus* species, maintained for more than 25 years in the laboratory of Entomology of the Benaki Phytopathological Institute, Kifissia, Greece. Adult mosquitoes are kept in wooden framed cages (33 cm × 33 cm × 33 cm) with a 32 × 32 mesh at 25 ± 2 °C, 80 ± 2% relative humidity and photoperiod of 14:10 (L:D) h. Cotton wicks saturated with 10% sucrose solution were used as food source. Females lay eggs in round, plastic containers (10 cm diameter × 5 cm depth) filled with 150 mL of tap water. Egg rafts were removed daily and placed in cylindrical enamel pans (with diameter of 35 cm and 10 cm deep), in order to hatch. Larvae reared under the same conditions of temperature and light and are fed daily with baby fish food (TetraMin, Baby Fish Food) at a concentration of 0.25 g L^{-1} of water until pupation. Then, the pupae were collected and introduced into the adult rearing cages.

2.5. Larvicidal bioassays

Stock solutions of tested EOs were prepared in ethanol and maintained in a freezer as 1% w/v solutions. They were dissolved in double distilled water to provide the solutions of the tested

Table 2
Essential oils yields.

Species (abbrev.)	Part distilled	Weight of aerial parts (g)	Volume of oil (mL)	Yield (mL/kg)
Anethum graveolens	Aerial parts (fresh)	260	1	3.9
Echinophora tenuifolia ssp. *sibthorpiana*	Aerial parts (fresh)	480	4.1	8.5
Chaerophyllum aromaticum	Aerial parts (fresh)	855	3.4	4.0
Cachrys ferulacea	Aerial parts (fresh)	270	1.3	4.8
Malabaila aurea	Aerial parts (fresh)	620	0.6	1.0